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TITLE: The Use of Venezuelan Equine Encephalitis Encoding the Her-2/neu Tumor Associated Antigen for the Prevention and Treatment of Breast Cancer

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**Overview:**

Our project has focused developing a vaccination protocol using Venezuelan Equine Encephalitis (VEE) replicons (VRP) encoding the Her-2/neu tumor associated antigen to mediate anti tumor immunity in Her-2/neu transgenic (Tg) mice. Initial work demonstrated that vaccination with the VRP encoding full-length Her2/neu elicited robust Her2/neu-specific cytotoxic T lymphocyte (CTL) reactivity in the non-Tg parental strain (FVB/n) of mice (Fig. 1). However, no significant Her2/neu-specific CTL reactivity was detected in cultures prepared from the FVB/neu Tg mice (Fig. 1). The results demonstrated that an element of self tolerance to Her2/neu exists in the transgenic mice. We postulated that due to the size of the cDNA encoding full length Her2/neu (greater than 4 kb), levels of *in vivo* expression were not sufficient to elicit a CD8<sup>+</sup> T cell response in FVB/neu Tg mice. Accordingly, a VRP encoding the extracellular and transmembrane domains (VRP-EC/TMD) spanning approximately 2 kb was established. More recently, a VRP encoding the intracellular domain (VRP-ICD) has also been generated. Notably, a comparable frequency of IL-2 and IFN $\gamma$  secreting T cells following stimulation with Her2/neu expressing NT2.5 tumor cells<sup>1</sup> was detected in cultures prepared from either FVB/n or FVB/neu Tg mice (Fig. 2). These results demonstrated that vaccination with VRP-EC/TMD could elicit significant Her2/neu-specific T cell reactivity despite tolerance to the neo-self antigen.

Next, the immunotherapeutic efficacy of VRP-EC/TMD was examined using a tumor challenge model. FVB/n or FVB/neu Tg mice were vaccinated with VRP-EC/TMD or left untreated and then injected s.c. with  $2 \times 10^6$  F-H2N1 expressing tumor cells in the hindquarter. This tumor cell line was derived from a spontaneously arising tumor in a FVB/neu Tg mouse and tested by FACS for neu expression regularly. As demonstrated in Fig. 3, a significant delay in tumor progression was detected in FVB/neu Tg mice vaccinated with VRP-EC/TMD but not in untreated mice. These results demonstrate that administration of VRP-EC/TMD can elicit T cell reactivity sufficient to delay tumor engraftment in FVB/neu Tg mice.

We've initiated four separate experiments examining the effect of VRP immunization on spontaneous tumor formation. The first pilot experiment was started over a year ago and has been completed (Figure 4A). These mice were immunized at 6 and 7 weeks of age and monitored for tumor development on a weekly basis. Normally, tumors first begin to appear around 7 months of age (200 days). Here we noticed a delay in the onset of tumor formation in the immunized FVB/neu Tg mice ( $p = 0.04$ , log rank). The second and third experiments were started concurrently, but with two different age groups of mice. The mice in experiment 2 (figure 4B) started treatment at 5 months of age to test the efficacy of late immunization, closer to the onset of tumor formation. While there is a visually obvious shift in the treatment group curve, it is not statistically significant. The third group of mice has been immunized 3 times at two-month intervals beginning at 8 weeks of age. The experimental group of 16 mice has been treated with EC-TMD VRP alone ( $n=8$ ) or EC-TMD and IL-12 encoding VRPs ( $n=8$ ). This experiment is still ongoing, however significant delays ( $p < 0.05$ , log rank) have been observed in the appearance of tumors in VRP-EC/TMD immunized mice relative to control FVB/neu Tg mice. These preliminary results suggest that VRP-EC/TMD can delay spontaneous mammary adenocarcinoma in FVB/neu Tg mice. Further experiments

have been initiated to examine the effect of Full length Her-2/neu, VRP-EC/TMD, VRP-ICD and an irrelevant VRP encoding the influenza hemagglutinin protein (HA) on spontaneous tumor formation and are still ongoing.

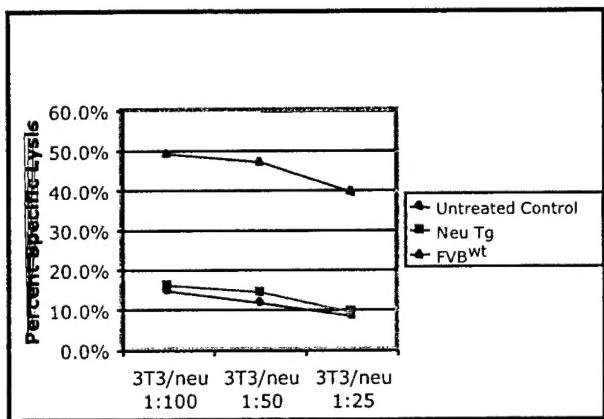
Our plans are to further establish the efficacy of VRP vaccination using a series of *in vitro* assays to determine the cellular phenotype mediating anti tumor protection in the FVB/*neu* Tg mice. The relative contribution of CD4<sup>+</sup> Th and CD8<sup>+</sup> CTL will be evaluated. We further plan to assess *in vivo* therapeutic efficacy using the *neu* expressing tumor challenge model and evaluate the effect of coimmunization with cytokine encoding VRP (and irrelevant replicon controls). This series of experiments will include vaccination prior to tumor cell engraftment as well as more clinically relevant experiments in which the ability of vaccination to mediate rejection post establishment will be evaluated. Finally, we plan to assess whether VRP immunization can prevent or inhibit the metastatic progression of transferred tumor to the lung. Spontaneous adenocarcinomas arising in FVB/*neu* Tg mice have been shown to metastasize to the lung and assay protocols have been developed in which cultured tumor cells are transferred to recipient mice I.V. and tumor engraftment in the lung is evaluated microscopically following india ink inflation of the lung.

### **Key Accomplishments:**

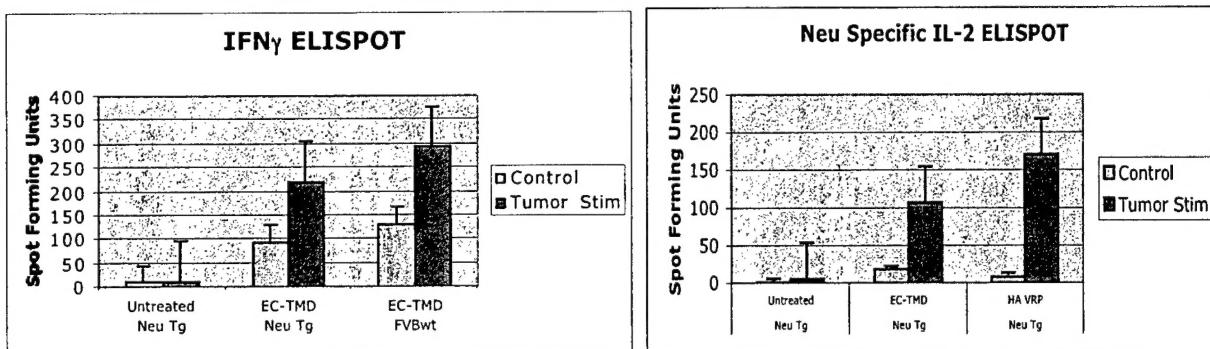
- Demonstrated CD8<sup>+</sup> CTL reactivity in FVB/n mice.
- Splenocytes from VRP immunized FVB/n and Her-2/*neu* Tg mice contain increased number of antigen specific IFN $\gamma$  producing cells.
- We have completed two tumor challenge experiments demonstrating the *in vivo* efficacy of Her-2/*neu* encoding VRP toward inhibiting the engraftment of transferred *neu* expressing tumor cells.
- Three experiments assessing the efficacy of Her-2/*neu* VRP vaccinations toward preventing spontaneous adenocarcinoma in Her-2/*neu* Tg mice have been completed. These results demonstrate VRP encoding Her-2/*neu* can delay spontaneous mammary adenocarcinoma.
- Completed *in vitro* testing and packaging of an additional truncated Her-2/*neu* encoding replicon expressing only the intracellular domain of the protein.

### **Reportable outcomes:**

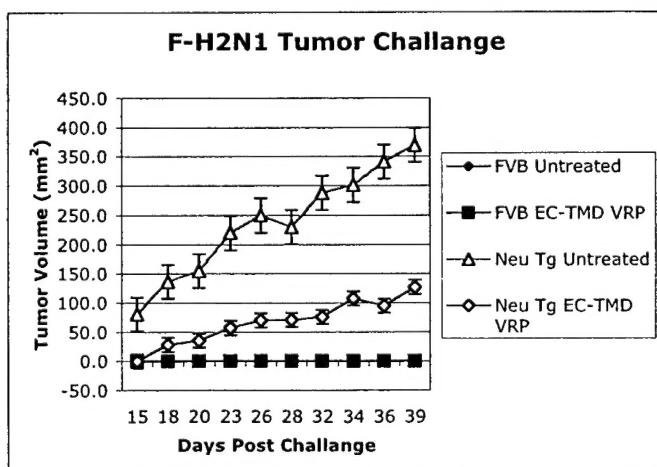
Results were presented at the Era of Hope Department of Defense Breast Cancer Research conference (oral communication and poster) in September of 2002.

**Figures:**

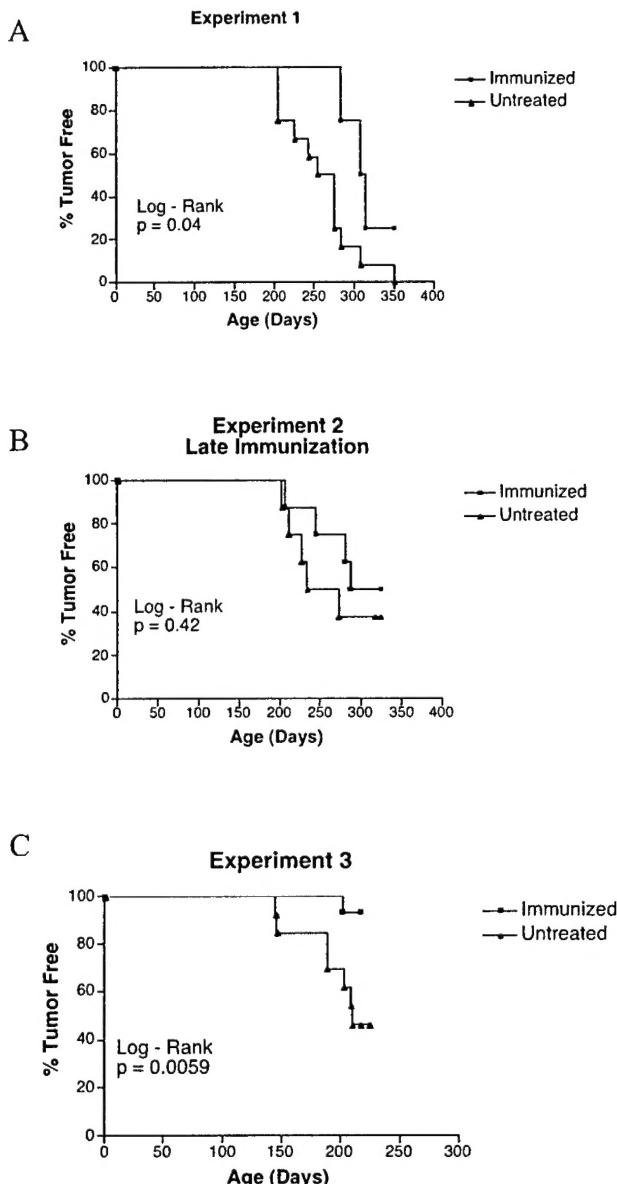
**Figure 1.** Standard 5 hr chromium release assay for Her-2/neu specific lysis. Wild type FVB and FVB/neu Tg mice were immunized I.P. 3 times at 7 day intervals with VRP-neu ( $5.0 \times 10^5$  I.U.). Splenocytes were prepared from immunized mice 10 days following the final injection and re-stimulated in vitro for 6 days with psorelen inactivated NIH-3T3 cells permanently transfected with a Her-2/neu encoding construct. Stimulated lymphocytes were incubated with  $^{51}\text{Cr}$  labeled targets in the indicated ratios for 5 hours.



**Figure 2.** Her2/neu-specific ELISPOT assay. FVB/n and/or FVB/neu Tg mice were immunized I.P. twice 2 weeks apart with  $5.0 \times 10^5$  I.U. VRP-EC/TMD and splenocytes were harvested 7 – 10 days later.  $7.5 \times 10^5$  (IFN $\gamma$ ) or  $1.0 \times 10^6$  (IL-2) splenocytes were cultured with  $1.0 \times 10^5$  mitomycin-C treated NT2.5 tumor cells for 24 hours. Cells were then aspirated and developed for spot forming units. For all treated mice,  $p < 0.05$  for tumor stimulated versus control.



**Figure 3.** Delayed tumor growth in VRP immunized mice. Wild type FVB and FVB/neu Tg mice were immunized I.P. twice at 10-day intervals with  $5.0 \times 10^5$  I.U. VRP-EC/TMD. One week later, mice received  $2.0 \times 10^6$  F-H2N1 tumor cells subcutaneously in the left flank. Challenged mice were monitored for tumor growth over a 39-day period and palpable tumors measured using a Vernier type caliper. Data is representative of two different experiments, four mice per group.  $P=0.037$  at day 32 (t-test) Untreated vs. immunized FVB/neu Tg mice.



**Figure 4.** Spontaneous tumor formation. Neu Tg mice were immunized with  $5.0 \times 10^5$  I.U. neu ECTMD VRP I.P. at 6 and 7 weeks of age (A), at five and 7 months of age (B) or at two, four and six months of age (C). Mice were visually monitored on a weekly basis for development of breast adenocarcinoma.

## Summary:

We have completed much of the work described for Specific Aim 1 in which we set out to establish and characterize VRP vaccination to induce Her-2/neu specific CD4<sup>+</sup> Th1 and CD8<sup>+</sup> CTL responses. We have completed all in vitro testing and packaging of replicons including two truncated Her-2/neu replicons encoding the extracellular and transmembrane domains, and encoding the intracellular domain of the protein. We have observed IL-2 and IFN $\gamma$  secreting cells in cultures prepared from vaccinated FVB/neu Tg mice indicating we could elicit significant Her-2/neu specific T cell reactivity despite

tolerance to the neo-self antigen. A H-2<sup>q</sup> restricted immunodominant epitope of Her-2/neu has recently been identified which should aid us in further characterizing the responding CD8<sup>+</sup> T cell population.<sup>2</sup>

For the second specific aim, we wished to determine the efficacy VRP vaccination for the prevention and/or treatment of mammary adenocarcinoma in FVB/*neu* Tg mice. We have made progress in evaluating the therapeutic efficacy of VRP toward preventing the progression of adenocarcinoma in mice challenged with F-H2N1 tumor cells. Specifically, we have demonstrated delayed onset and inhibited growth in challenged mice immunized with the truncated VRP-EC/TMD. We have begun to evaluate the efficacy of Her-2/*neu* encoding VRP in preventing the spontaneous development of mammary adenocarcinoma in FVB/*neu* Tg mice. These results demonstrate vaccinated mice display a delay in the development of spontaneous mammary adenocarcinoma as compared to untreated control mice.

Comparative analyses are ongoing to determine the magnitude of CD4<sup>+</sup> and CD8<sup>+</sup> T cell reactivity induced in FVB/*neu* Tg mice following vaccination with VRP-EC/TMD plus/minus cytokine encoding VRPs, in addition to VRP-ICD (plus/minus cytokine VRPs). Experiments will examine the efficacy of VRP treatment *after* FVB/*neu* Tg mice are challenged with tumor cells. Depending on their adjuvant effect, VRP encoding the appropriate cytokine will be co-administered with VRP-EC/TMD (and VRP-ICD) and the efficacy of this regime to suppress the progression of established tumors in the challenge model will be investigated. Studies will continue to evaluate the immunotherapeutic efficacy of VRP-EC/TMD (and VRP-ICD) in the prevention of spontaneous tumor formation in FVB/*neu* Tg mice. Finally, we have established adenoviral-associated viral (AAV) recombinants encoding the EC/TMD and ICD of Her2/*neu*. Once packaged, T cell reactivity induced following vaccination with the AAV vectors will be characterized.

## References:

1. **R. Todd Reilly**, Morris B. C. Gottlieb, Anne M. Ercolini, Jean-Pascal H. Machiels, Colin E. Kane, Francesca I. Okoye, William J. Muller, Katherine H. Dixon, and Elizabeth M. Jaffee. **2000**. HER-2/*neu* Is a Tumor Rejection Target in Tolerized HER-2/*neu* Transgenic Mice. *Cancer Research* 60, 3569-3576
2. **Anne M. Ercolini**, Jean-Pascal H. Machiels, Yi Cheng Chen, Jill E. Slansky, Martin Giedlen, R. Todd Reilly, and Elizabeth M. Jaffee. **2003**. Identification and Characterization of the Immunodominant Rat HER-2/*neu* MHC Class I epitope Presented by Spontaneous Mammary Tumors from HER-2/*neu*-Transgenic Mice. *The Journal of Immunology* 170: 4273-4280